From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 December 1999 (02.12.99)	
International application No. PCT/GB99/00999	**Applicant's or agent's file reference** P/4168.WO
International filing date (day/month/year) 31 March 1999 (31.03.99)	**Priority date** (day/month/year) 17 April 1998 (17.04.98)
Applicant DURRANT, James, Robert et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 November 1999 (16.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia RANAIVOJAONA

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P/4168.WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/00999	31/03/1999	17/04/1998
International Patent Classification (IPC) or national classification and IPC G01N27/327		
Applicant IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICI		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/11/1999	13.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Oechsner de Coninck Telephone No. +49 89 2399 2379

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/00999

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10	as originally filed	

Claims, No.:

22-31	as originally filed	
1-21	with telefax of	23/06/2000

Drawings, sheets:

1/3-3/3	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1. Novelty of claims 1-21: Art.33(2) PCT:

None of the documents of the International Search Report discloses a biosensor comprising a nanocrystalline metal oxide semiconductor film having proteins immobilised thereon or a process to produce such biosensor. The subject matter of claims 1-21 therefore meets the requirements of Art.33(2) PCT.

V.2. Lack of inventive step of product claims 1-16 and process claims 17-21: Art.33(3) PCT:

Claims 1,2:
The closest prior art document is reflected by D3 (WO 96/00198) cited on p.1, last § of the present description. This document discloses a nanocrystalline titanium dioxide film and its production (see, p.1, last § to p.2, penultimate §). According to p.3, lines 4,5, this film may be used as sensor to immobilise enzymes and catalysators. The term "protein" used in the present application however includes enzymes and catalysators (see, present p.3, lines 9-12, claim 8). Furthermore, claim 1 referring only to "nanocrystalline titanium dioxide film", no differences can be seen between the composition of the present film and that disclosed by D3. It may also be added that the term "at least one protein immobilised on at least one portion of said film" is very broad. Hence, the subject matter of claims 1,2 does not appear to involve an inventive step in the sense of Art.33(3) PCT in view of this disclosure.

Claims 3-16:
The features of claims 3 and 4 are derivable from D3, p.4, claim 2.
The features of claims 5-16 are not surprising in the present technical field of biosensors.
Hence, the subject matter of claims 3-16 does not involve an inventive step (Art.33(3) PCT).

Claims 17-21:

The process steps described by present claim 17 are common in the present technical field of biosensors. According to the statement in above § V.2 relating to claims 1,2, the subject matter of process claim 17 does not meet the requirements of Art.33(3) PCT. The features of claims 18-21 being not surprising in the present technical field, they do not amount to an inventive step in combination with those of claim 17.

Re Item VII

Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in document D1 is not mentioned in the description, nor is this document identified therein.

Re Item VIII

Certain observations on the international application

The description is not in conformity with the amended claims. All parts describing immobilisation of chemical species other than proteins should be deleted.

PATENT COOPERATION TREATY

SOUTHAMPTON
23 JUL 1999
JAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
D. YOUNG & CO.
Attn. TURNER, James A.
21 New Fetter Lane
London EC4A 1DA
UNITED KINGDOM

Date of mailing *(day/month/year)*	21/07/1999
Applicant's or agent's file reference: P/4168.WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/GB 99/00999	International filing date *(day/month/year)* 31/03/1999
Applicant: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICI	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Jaap Hurenkamp

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
P/4168.WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 00999	31/03/1999	17/04/1998
Applicant		
IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICI		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTER IONAL SEARCH REPORT

International Application No
PCT/GB 99/00999

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/327 C12Q1/00 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 585 646 A (KOSSOVSKY NIR ET AL) 17 December 1996 see column 6, line 24 - column 6, line 54 ---	1,26
X	GERFIN, T. ET AL: "Molecular and supramolecular surface modification of nanocrystalline TiO2 films: charge-separating and charge-injectin devices" PROG. INORG. CHEM. (1997), 44(MOLECULAR LEVEL ARTIFICIAL PHOTOSYNTHETIC MATERIALS), 345-393 CODEN: PIOCAR;ISSN: 0079-6379, XP002108197 see the whole document ---	1,26
A	WO 92 21976 A (FISONS PLC) 10 December 1992 see the whole document ---	1,26

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1999	21/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 364 797 A (OLSON DAVID H ET AL) 15 November 1994 see column 1, line 5 - column 4, line 56	1,26
A	EP 0 596 421 A (HOFFMANN LA ROCHE) 11 May 1994 see abstract	1,26
A,P	US 5 874 047 A (FROHNHOFF STEPHAN ET AL) 23 February 1999 see abstract	1,26
A	WO 96 00198 A (PENTH BERND) 4 January 1996 cited in the application see the whole document	1,26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/00999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5585646 A	17-12-1996	US 5506420 A	09-04-1996
WO 9221976 A	10-12-1992	EP 0643833 A	22-03-1995
		JP 6507709 T	01-09-1994
US 5364797 A	15-11-1994	NONE	
EP 0596421 A	11-05-1994	CA 2108705 A	07-05-1994
		JP 6265553 A	22-09-1994
US 5874047 A	23-02-1999	DE 4427921 A	15-02-1996
		CA 2196895 A	22-02-1996
		WO 9605512 A	22-02-1996
		EP 0775314 A	28-05-1997
		JP 10504388 T	28-04-1998
WO 9600198 A	04-01-1996	DE 4421978 A	04-01-1996
		DE 4437767 A	25-04-1996
		DE 4439722 A	15-05-1996
		EP 0766657 A	09-04-1997
		US 5885657 A	23-03-1999

SN

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 27/327, C12Q 1/00, G01N 33/543 — A1

(11) International Publication Number: WO 99/54718

(43) International Publication Date: 28 October 1999 (28.10.99)

(21) International Application Number: PCT/GB99/00999

(22) International Filing Date: 31 March 1999 (31.03.99)

(30) Priority Data:
9808264.7 17 April 1998 (17.04.98) GB

(71) Applicant *(for all designated States except US)*: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE [GB/GB]; Sherfield Building, Exhibition Road, London SW7 2AZ (GB).

(72) Inventors; and

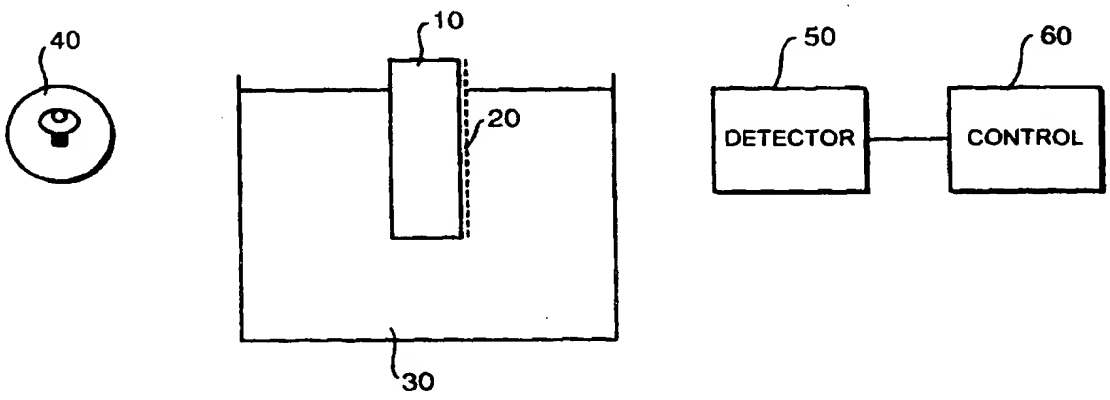
(75) Inventors/Applicants *(for US only)*: DURRANT, James, Robert [GB/GB]; 44 Standen Road, London SW18 5TQ (GB). CASS, Anthony, Edward, George [GB/GB]; 39 Novello Street, Parsons Green, London SW6 4JB (GB). GILARDI, Gianfranco [IT/GB]; 49 Wimbledon Park Road, London SW18 5SJ (GB).

(74) Agent: TURNER, James, Arthur; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: BIOCHEMICAL DEVICES AND THEIR METHODS OF MANUFACTURE



(57) Abstract

Biochemical devices comprising a sensing surface that is at least partially covered by a nanocrystalline metal oxide semiconductor film (20) which provides a recipient surface for immobilising biochemical species on. The film (20) has a mesoporous surface which gives up to a 150 increase in biochemical species adsorption when compared to a flat surface. The biochemical devices comprising these surfaces can be optical and electrochemical biosensors and reactors for synthetic or biodegradation reactions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BIOCHEMICAL DEVICES AND THEIR METHODS OF MANUFACTURE

This invention relates to biochemical devices such as biosensors and their methods of manufacture.

A wide range of devices used in chemistry and biology (such as in the biotechnological field) require the immobilisation of a biochemical species upon a substrate or film, so that the biochemical species can be sensed or can react with another substance. Such devices include electrochemical, optical and electro-optical bioanalytical devices, and reactors for synthetic or biodegradation reactions. These reactors may be driven optically and/or electrically.

A range of strategies are currently employed for the immobilisation of biochemical species in biochemical devices, the strategy used depending upon the device and its application. For example, an electrochemical biosensor device requires electrical contact between the biochemical species, such as a protein, and a conducting electrode. Procedures employed in optimising this contact include aligning the biochemical species on chemically modified electrodes, attaching electron-transporting groups or modified redox co-factors and immobilising the biochemical in polymer matrices. With optical biosensors, however, optical transparency of the solid substrate is a key issue. In these devices polymeric or silicate glass matrices have been employed to encapsulate the biochemical species. These biosensors are used to detect a wide variety of different things, such as sugars or pH.

In many cases, the immobilisation of the biochemical species is achieved during the process of matrix formation. Such matrix formation requires drying for prolonged periods and / or at elevated temperatures. Such procedures tend to cause denaturisation of many biochemical species, in particular proteins.

WO-A-96/00198 discloses a process for producing ceramic layers including titanium dioxide. These ceramic layers can have enzymes immobilised in them for use in the biochemical field. These layers are produced by mixing a suspension containing TiO_2 and an enzyme and then drying it in a stream of warm air at 80°C. Due to the high temperature used in drying this process is only applicable to thermally stable biochemical species. Many biochemical species are not thermally stable.

In accordance with one aspect of the present invention there is provided a biochemical device comprising a surface for immobilising a biochemical species, wherein said surface is at least partially covered with a nanocrystalline metal oxide semiconductor film, said film providing a recipient surface for immobilisation of said biochemical species.

Thus the present invention alleviates the disadvantages of the prior art by the use of nanocrystalline metal oxide semiconductor films. These films typically comprise nanometer-sized crystalline particles (typical diameter 5 - 50 nm) which are densely packed to form a mesoporous structure with a surface area up to 1000 times greater than its geometrical area.

In other words, in embodiments of the invention a biomolecule can be immobilised on a preformed, mesoporous film, in contrast to previous techniques of matrix immobilisation where film formation and biomolecule immobilisation are achieved in a single process. This allows the immobilisation to be conducted under conditions which do not denature the protein or other biomolecule, of which the use of lower temperatures is one important example. This combination of mild immobilisation conditions and the specific properties of the film (high biomolecule loading, optical transparency, stability, electrical conductivity) are technical advantages of this invention.

Furthermore, these nanocrystalline metal oxide semiconductor films have simple and flexible biochemical attachment chemistries. Attachment may occur covalently, by adsorption, by bio-derivitisation of the film or by combinations thereof.

Nanocrystalline metal oxide semiconductor films combine a high surface area and excellent stability with efficient current transport. In addition to a high surface area these substances have rapid diffusion paths due to the pores being larger than the biochemical species, and this in turn allows the rapid mass transport of the analyte into and through the film. Furthermore, their high surface area to geometrical area enables a small device to hold a large quantity of the biochemical species. This enables the size of the device to be decreased resulting in increased mass transport giving faster response times. Additionally a smaller device has the practical advantage of being able to be used in restricted volumes. Furthermore, the high loading of the biochemical molecules

makes them less susceptible to loss of activity. No existing materials employed for biochemical applications exhibit all of these properties.

In a preferred embodiment the nanocrystalline metal oxide semiconductor is titanium dioxide, TiO_2. TiO_2 has a wide band gap and as such is optically transparent, making it suitable for optical applications as well as electrical ones.

In further embodiments the nanocrystalline metal oxide semiconductor is zinc oxide, ZnO or zirconium dioxide, ZrO_2.

In one embodiment a biochemical species is immobilised on at least a portion of the film. Preferably, the biochemical species is a protein. The term protein, when used in this application should be taken to include enzymes, antibodies or fragments thereof and other polypeptides capable of binding molecules or catalysing their transformation to another molecular species.

In a further embodiment attachment of the biochemical species occurs by bio-derivitisation of the film. Bio-derivitisation of the film involves using a chemical species - such as a biomolecule - as an intermediary, the biochemical species molecule becoming immobilised to the film via the chemical species - i.e. the biochemical species is bound to the chemical species which is in turn bound to the film. This has the advantage of increasing the number of possible biochemical species that can be immobilised by the film, and improving the stability of immobilisation. An example of this is the use of the enzyme avidin. Avidin is expected to bind strongly to TiO_2 due to its positive charge. Any biomolecule with a biotin group attached (readily added) can bind to avidin.

In a further embodiment the biochemical device is a biosensor. The nanocrystalline metal oxide semiconductor film providing an ideal surface for immobilising a sensing biochemical species for use in such a device.

Advantageously, the film forms an array on the surface. A conveniently shaped sensing area can thus be formed. Furthermore, the array allows for different sensing biochemical species to be attached to different portions of the array. Thus a variety of substances can be detected and depending on the biochemical species used, both electrochemical and optical signals may be produced. These arrays may be simply and accurately produced by screen printing or other method compatible with the properties of the substance being deposited.

In a preferred embodiment a pH sensitive dye is additionally attached to a further portion of the film. Thus changes in the sample pH can be monitored optically and the results can be used to correct for pH effects on, for example, an enzyme-based sensing element.

In one embodiment the biosensor is an electrochemical biosensor, comprising an electrical circuit connected to the film, the circuit comprising a meter for monitoring changes in the current, voltage, conductivity or impedance in the circuit produced by an electrochemical reaction. The conductive nature of the film makes it especially suited to such a device.

In a further embodiment the biosensor comprises an optical sensor that acts to optically detect substances, by monitoring the interaction of electromagnetic radiation with the molecules present. The transparent nature of many metal oxide semiconductor films makes them particularly suited to such an application. In preferred embodiments the immobilised biochemical species is a fluorescent labelled or fluorophore labelled biochemical species and it is the fluorescence thus produced that provides an indication of the concentration of the substance under investigation. Control electronics form part of the device and are used to calculate the concentration. Alternatively, the fluorescence may arise from the binding of a fluorescent molecule to a biochemical species already on the surface. Fluorescence may also be generated by the formation of a fluorescent product from a non-fluorescent substrate through an enzymatic reaction.

In one embodiment the device comprises both an electrochemical biosensor and an optical one, such that a plurality of substances may be detected by the one sensor. The conductive and transparent nature of the film makes it particularly suitable for use in such a dual purpose environment.

In a further preferred embodiment the sensing biochemical species can be electrochemically or photochemically switched to a reactive state by oxidation or reduction or through the production of small molecules or ions e.g. H^+. This allows the sensing element in the device to be regenerated by switching the direction of the electric current after optical sensing. Furthermore, where there are concerns about the stability of the sensing molecule the current measured during the electrochemical regeneration step

would be an indication of the amount of active material present and so give an opportunity for recalibration.

In a preferred embodiment the biosensor comprises a photoelectric element or other power generating element such that the biosensor can be used in remote areas, for military applications and for long term sensing, with data being sent by telemetry. Advantageously the photoelectric element may be a portion of the TiO_2 film, its photovoltaic properties acting to produce the necessary power.

In a further embodiment the biochemical device is a reactor for synthetic, catalytic or biodegradation reactions. The film provides a suitable site for immobilising biochemical species, in particular, enzymes involved in the reaction. In one embodiment the device comprises an electrical source for electrically driving the reaction, the electrical conductivity of the film making it particularly suitable for such an arrangement. In another embodiment the biochemical device comprises an optical source, the reaction being driven optically. The transparent nature of many nanocrystalline metal oxide semiconductor films makes them particularly suited to such an application.

In a preferred embodiment the biochemical reactor comprises a photoelectric element for producing the reaction driving current. Advantageously the nanocrystalline metal oxide semiconductor film may be TiO_2, and the photoelectric element may be a portion of that TiO_2 film, its photovoltaic properties acting to produce a photoelectric current.

In some embodiments the reaction may be optically driven, the biochemical device being arranged to receive electromagnetic radiation, possibly by the provision of optically transparent windows in the outer casing of the biochemical device. Alternatively, the biochemical device may include a light source.

In accordance with another aspect of the present invention there is provided a method of manufacturing a biochemical device, comprising covering at least a portion of a sensing surface with a film of nanocrystalline semiconductor, contacting said film with a biochemical species such that said biochemical species is immobilised onto said film. The immobilisation is preferably achieved after fabrication of the semiconductor film, under conditions which may be selected to minimise or at least reduce degradation/denaturisation of the biochemical species.

Preferably, the film is applied by screen printing followed by sintering in air. Nanocrystalline metal oxide semiconductors are particularly suited to screen printing as they form colloidal suspensions. Screen printing is a well established and cheap technology providing films from low cost precursors. Such means of fabrication also enables robust films of the material to be deposited in various patterns.

In some embodiments the biochemical species are caused to contact the preformed film by immersing said at least partially covered sensing surface into an aqueous solution of a biochemical species such that said biochemical species is immobilised onto said film. This immobilisation may be achieved without the use of non-physiological temperatures, pH and solvents.

The generic nature of the immobilisation chemistry onto the nanocrystalline material, in particular through adsorption or covalent attachment, means the deposition of the biochemical species can advantageously be done using a commercially available "gridding robot". This is an instrument which allows volumes of liquids to be dispensed at specified x-y co-ordinates. Different liquids (e.g. biomolecules in solution) can be dispensed in an arbitrary pattern. The advantage is that once the pattern of sensing elements has been laid down by printing the biomolecules can be patterned on top using the robot. In alternative embodiments other deposition methods such as ink jet printing may be used.

In preferred embodiments the temperature at which the film is contacted with the biochemical species is 4°C, in order to optimise stability of the biochemical species.

In some embodiments the biochemical species is a protein.

Embodiments of the present invention will now be described, by way of example only, and with reference to the accompanying drawings, in which:

Figure 1 illustrates a biosensor according to an embodiment of the present invention;

Figure 2 illustrates the fluorescent emission spectra of IANBD labelled maltose binding protein coated TiO_2 films immersed in maltose and sucrose solutions;

Figure 3 illustrates an electro-optical biosensor;

Figure 4 illustrates absorption spectra of cytochrome c coated TiO_2 films before and after application of -0.6V vs Ag/Ag cl (reference electrode); and

Figure 5 illustrates photochemical reduction of cytochrome-c showing absorption spectra of cytochrome C/TiO_2 films before (-) and after (....) ultraviolet illumination of the film.

Figure 1 illustrates a fluorescent biosensor for sensing the presence of maltose. The biosensor comprises a substrate 10 which is covered by a film 20 of TiO_2 with a IANBD (4-[N-(2-(iodoacetoxy)ethyl)-N-methylamino]-7-nitobenz-2oxa-1,3diazole) labelled Maltose Binding Protein (MBP) immobilised on it, a container for holding the solution 30 under investigation, a light source 40, a fluorescence detector 50 and control electronics.

The coated substrate was produced by screen printing a 10μm thick nanocrystalline TiO_2 film onto the substrate using a colloidal suspension of TiO_2 , and then immersing the substrate in an aqueous solution of a IANBD labelled Maltose Binding Protein (MBP) at 4°C. This results in an approximate monolayer coverage of the film with MBP. This coverage is up to a 1000 fold increase in adsorption relative to a flat surface due to the mesoporous structure of the film.

The biosensor operates by immersing the MBP covered substrate in the solution 30 under investigation. Any maltose present in the solution will bind to the MBP thereby increasing the fluorescence of the label by up to 200%. The substrate is illuminated by a light source 40 at an appropriate wavelength and the fluorescence is detected by a fluorescence detector 50, the detection being aided by the optical transparency of the film. Control electronics 60 calculate the amount of maltose present in the solution from the fluorescence detected, and output the result.

Alternatively, the generic nature of the immobilisation chemistry onto the nanocrystalline material, in particular through adsorption or covalent attachment, means the deposition of the biochemical species can advantageously be done using a commercially available "gridding robot". This is an instrument which allows volumes of liquids to be dispensed at specified x-y co-ordinates. Different liquids (e.g. biomolecules in solution) can be dispensed in an arbitrary pattern. The advantage is that once the pattern of sensing elements has been laid down by printing the biomolecules can be patterned on top using the robot. In alternative embodiments other deposition methods such as ink jet printing may be used.

In other embodiments, a fluorophore-labelled species could be used.

Figure 2 illustrates the results produced by the device illustrated in Figure 1, for a solution containing 500μM maltose and one containing sucrose. As expected the solution containing maltose causes the fluorescence intensity to increase, whereas the control solution containing only sucrose shows no change in fluorescence intensity.

Figure 3 illustrates an electro-optical biosensor, comprising a substrate 10 which is covered by a film 20 of TiO_2 with cytochrome c immobilised on it. The substrate is connected to a variable voltage supply 70. An absorption spectrometer is shown schematically as a light source 45 and a detector 55, but the actual implementation of such a spectrometer to provide an absorption spectrum through the biosensor is well known in the art. A detector can be connected in the circuit to monitor changes in the current and/or voltage in the circuit produced by an electrochemical reaction taking place.

The absorption spectra illustrated in Figure 4 are produced by the device illustrated in Figure 3. The two spectra are produced by the cytochrome c before and after the application of -0.6V to the back surface of the substrate respectively. The cytochrome c protein was immobilised on the TiO_2 coated substrate by immersion of the substrate in an aqueous solution of cytochrome c at 4° C.

Figure 4 shows changes in the characteristic reduction of the cytochrome c with applied voltage and it is therefore clear that there is electrical connectivity between the external circuit and the adsorbed protein. These results thereby demonstrate the suitability of a substrate coated with a TiO_2 film for use in an electrochemical biosensor.

Furthermore, such a biosensor can be electrochemically switched to a reactive state by an applied voltage that aids oxidation or reduction. This allows the sensing element in the device to be regenerated by switching the direction of the electric current after optical sensing. It has further been demonstrated that the immobilised cytochrome c may be reduced by ultraviolet illumination. Illumation was achieved by 337nm pulses from a nitrogen laser, resulting in band gap excitation of the TiO_2 film. Thus the redox states of immobilised proteins may be modulated by electromagnetic illumination, resulting from either a separate photoelectric element or from solar irradiation. Thus the sensing element in the device may be regenerated by either electrical current or electromagnetic irradiation. Such reduction/oxidation of the biomolecule is also

applicable to the function of electrocatalytic/photocatalytic biochemical devices for synthetic, bioremediation and other biotechnological applications.

Figure 5 illustrates the photochemical reduction of Fe(III) Cyt-*c* to Fe(II) Cyt-*c* driven by 337nm bandgap excitation of the TiO_2. This reduction is attributed to photoinduced electron transfer from the conduction band of the TiO_2 to the immobilised protein. This demonstrates that TiO_2/boimolecule devices arranged for synthetic/catalytic/biodegradation reactions can be driven optically as well as electrically. This photochemical reduction (or, in principle oxidation) could also be used to regenerate the sensing state during biosensor function.

Table 1, below, gives a summary of some of the proteins that have been successfully immobilised on TiO_2 nanoporous films.

Protein	Source	Activity	Comments	Activity
Cytochrome c	Mammalian	Electron transfer	Electrochemically coupled to TiO_2	✓
Maltose binding protein	Bacteria (recombinant)	ligand binding	Ligand binding detected by fluorescence	✓
Cytochrome c peroxidase	Yeast (recombinant)	Hydrogen peroxide reduction		Not tested
Haemoglobin	Mammalian	O_2 binding	Electrochemically coupled to TiO_2. Can be used as an NO sensor.	✓
Alkaline phosphatase	Bacteria (recombinant)	Hydrolysis of Phosphate esters	Activity measured by fluorescence. Both wild-type and "his tag" proteins can be immobilised. The latter after Ni^{2+} treatment.	✓
Horseradish peroxidase	Plant	Substrate oxidation	Activity measure by absorbance	✓

Table 1

The above examples illustrate a key advantage of using a substrate coated with a TiO_2 film, namely that immobilisation of the biochemical species is achieved at 4°C, thereby reducing the risks of denaturisation. Another advantage of using a TiO_2 film is that in some embodiments a portion of the film may be used as a photoelectric element, allowing the device to be used in remote locations without a separate power source.

In other embodiments of the present invention other nanocrystalline semiconductor films, such as ZnO or ZrO are used.

In further embodiments the nanocrystalline metal oxide semiconductor film is in the form of an array which is screen printed onto the surface. Different biochemical species may be attached to different portions of the array and in some embodiments a pH sensitive dye is also applied to the surface.

Other embodiments of the present invention include the use of nanocrystalline semiconductor metal oxide films in reactors for synthetic or biodegradation reactions. These reactors can be electrically or optically driven.

In further embodiments a pH sensitive dye is additionally attached to a further portion of the film. Thus changes in the sample pH can be monitored optically and the results can be used to correct for pH effects on, for example, an enzyme-based sensing element.

It will be apparent, of course, that the present invention has been described above by way of example only and that modifications may be made within the scope of the appended claims.

CLAIMS

1. A biochemical device comprising a surface for immobilising a biochemical species, wherein said surface is at least partially covered with a nanocrystalline metal oxide semiconductor film, said film providing a recipient surface for immobilisation of said biochemical species.

2. A biochemical device according to claim 1, wherein said nanocrystalline metal oxide is titanium dioxide.

3. A biochemical device according to claim 1, wherein said nanocrystalline metal oxide is zinc oxide.

4. A biochemical device according to claim 1, wherein said nanocrystalline metal oxide is zirconium dioxide.

5. A biochemical device according to any of claims 1 to 4, comprising at least one biochemical species immobilised on at least a portion of said film.

6. A biochemical device according to claim 5, wherein said biochemical species is a protein.

7. A biochemical device according to any of claims 1 to 6, wherein the film further comprises biomolecules immobilised on it, said biomolecules being adapted for attachment by a biochemical species.

8. A biochemical device according to any of claims 1 to 7, wherein said biochemical device is a biosensor.

9. A biosensor according to claim 8, wherein said film forms an array on said surface.

10. A biosensor according to claim 9, wherein different biochemical species are bound to different portions of the array.

11. A biosensor according to any of claims 8 to 10, wherein a further portion of said surface is coated with a pH sensitive dye.

12. A biosensor according to any of claims 8 to 11, wherein said biosensor is an electrochemical biosensor, further comprising an electrical circuit electrically connected to said film, said circuit comprising a detector for monitoring changes in the current or voltage in the circuit produced by an electrochemical reaction.

13. A biosensor according to any of claims 8 to 11, wherein said biosensor is an optical biosensor, further comprising an optical sensor for monitoring a reaction by sensing the interaction of electromagnetic radiation with the molecules present.

14. An optical biosensor according to claims 5 and 13, wherein said at least one immobilised biochemical species is a fluorescent or fluorophore labelled biochemical species, said film is optically transparent, and said biosensor further comprises a light source and control electronics for calculating concentrations from the output of said optical sensor.

15. A biosensor according to any of claims 8 to 11, further comprising an electrical circuit electrically connected to said film, and an optical sensor.

16. A biosensor according to claim 15, wherein said immobilised biochemical species is such that it can be electrochemically or photochemically switched to a sensing state by oxidation or reduction, the results of the sensing reaction being measured optically or electrically.

17. A biosensor according to any of claims 8 to 16, wherein said biosensor further comprises an element for supplying power to said biosensor.

18. A biosensor according to claim 17, wherein said power supplying element comprises a photoelectric element operable to supply power to said biosensor in response to electromagnetic radiation.

19. A biosensor according to claims 2 and 18, wherein a portion of said TiO_2 film forms said photoelectric element.

20. A biochemical device according to any of claims 1 to 7, wherein said biochemical device is a reactor for synthetic, catalytic or biodegradation reactions.

21. A biochemical device according to claim 20, further comprising an electrical source electrically connected to said film, said reaction being driven electrically.

22. A biochemical device according to claim 21, wherein said electrical source comprises a photoelectric element.

23. A biochemical device according to claims 21 and 22 wherein a portion of said TiO_2 film forms said photoelectric element.

24. A biochemical device according to claim 20, said device being operable to receive external radiation in order to optically drive said reaction.

25. A biochemical device according to claim 20, further comprising a light source, said reaction being driven optically.

26. A method of manufacturing a biochemical device, comprising covering at least a portion of a sensing surface with a film of nanocrystalline semiconductor, contacting said

preformed film with a biochemical species such that said biochemical species is immobilised onto said film.

27. A method of manufacturing a biochemical device according to claim 26, wherein said film of nanocrystalline semiconductor is applied to said sensing surface by screen printing.

28. A method of manufacturing a biochemical device according to claim 26 or 27, wherein said preformed film is contacted with a biochemical species by immersion of said at least partially covered surface in an aqueous solution of the biochemical species.

29. A method of manufacturing a biochemical device according to claim 26 or 27, wherein the biochemical species is deposited on the film using a gridding robot, or other dispensing device such as an ink-jet printer.

30. A method of manufacturing a biochemical device according to any of claims 26 to 29, wherein the temperature at which the film is contacted with the biochemical species is substantially 4°C.

31. A method of manufacturing a biochemical device according to any of claims 26 to 30, wherein said biochemical species is a protein.

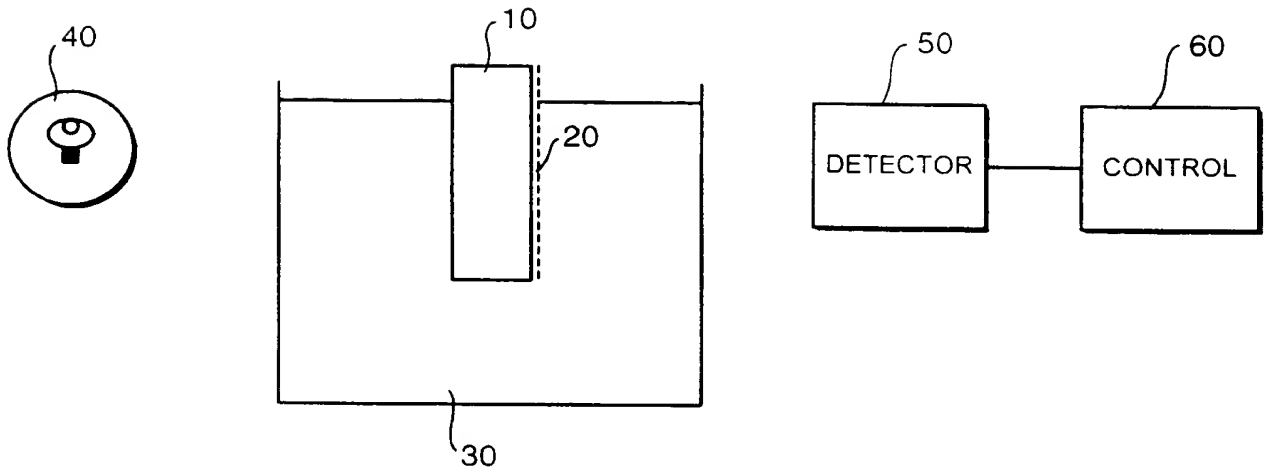

40
10
20
30
50
60
DETECTOR
CONTROL

FIG.1

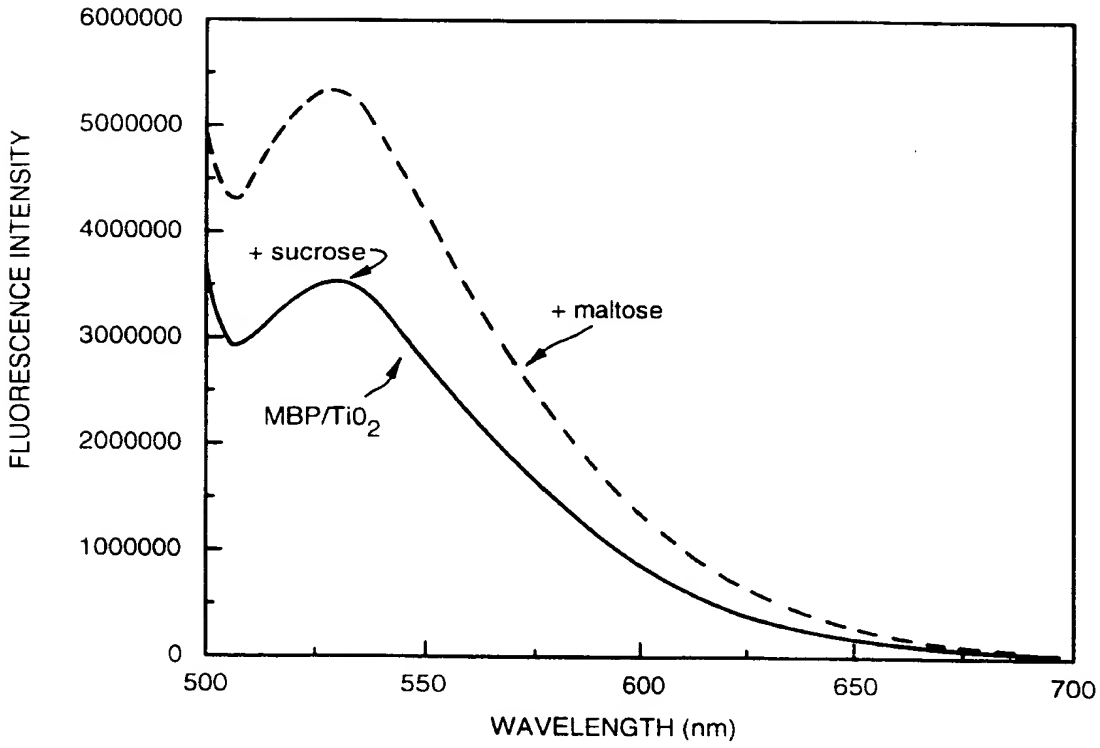

6000000
5000000
4000000
3000000
2000000
1000000
0
FLUORESCENCE INTENSITY
+ sucrose
+ maltose
MBP/Ti0$_2$
500
550
600
650
700
WAVELENGTH (nm)

FIG.2

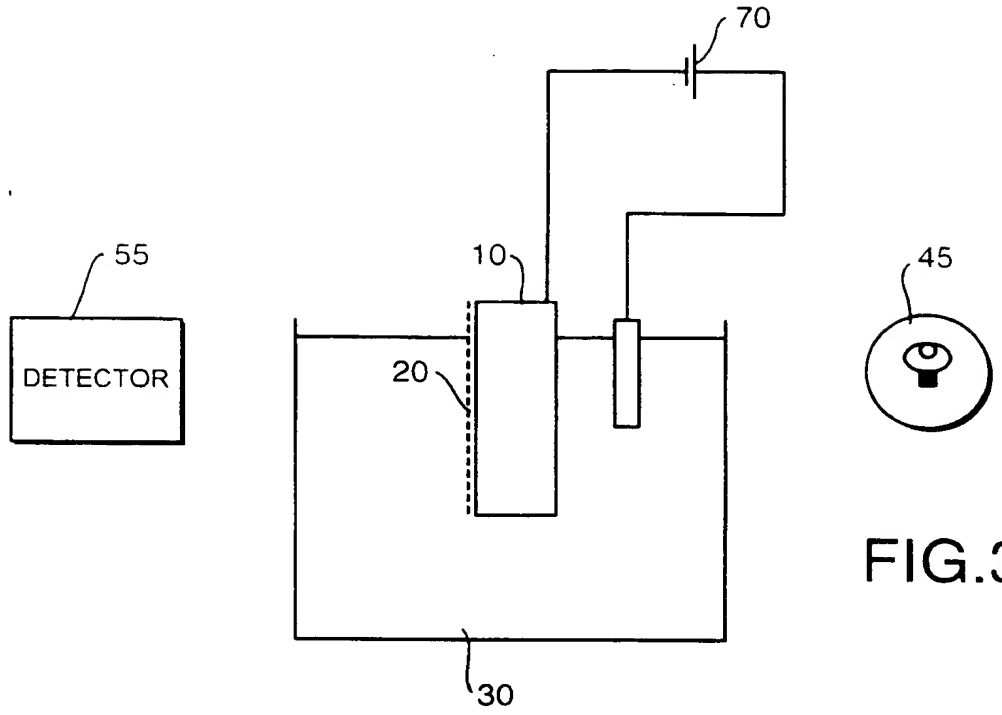

70
55
10
45
DETECTOR
20
30

FIG.3

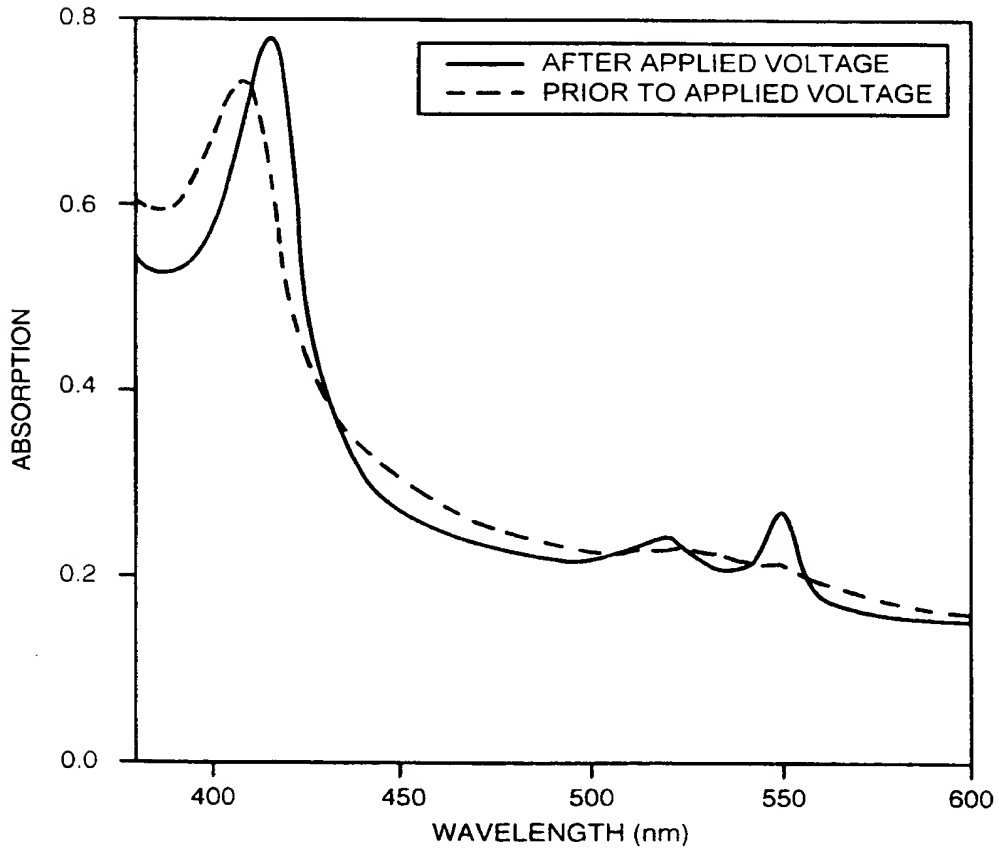

0.8
0.6
0.4
0.2
0.0
ABSORPTION
AFTER APPLIED VOLTAGE
PRIOR TO APPLIED VOLTAGE
400
450
500
550
600
WAVELENGTH (nm)

FIG.4

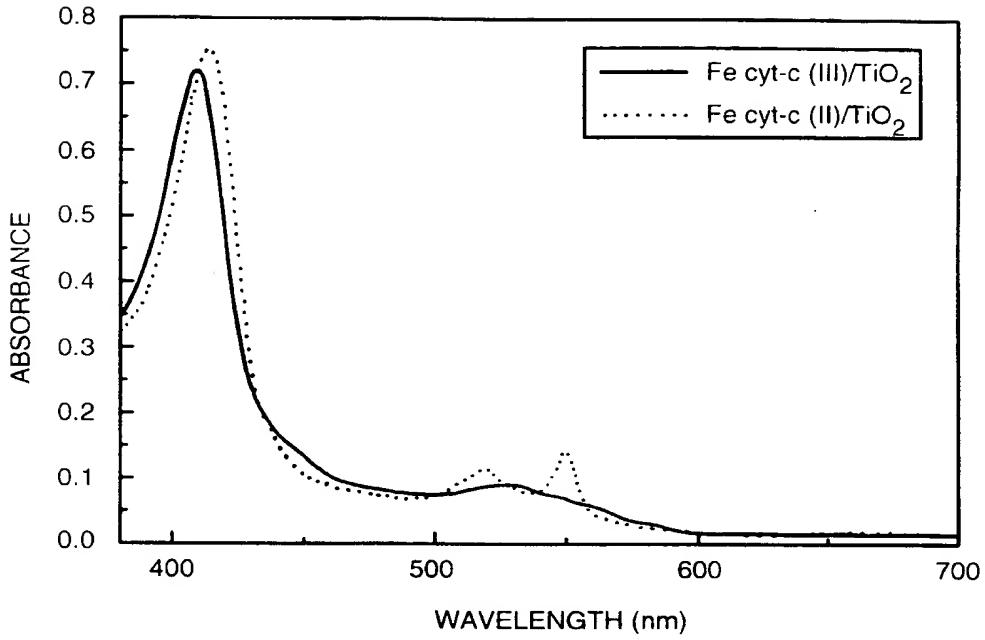

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ABSORBANCE
400
500
600
700
WAVELENGTH (nm)
Fe cyt-c (III)/TiO2
Fe cyt-c (II)/TiO2

FIG.5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/00999

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/327 C12Q1/00 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 585 646 A (KOSSOVSKY NIR ET AL) 17 December 1996 see column 6, line 24 - column 6, line 54 ---	1,26
X	GERFIN, T. ET AL: "Molecular and supramolecular surface modification of nanocrystalline TiO2 films: charge-separating and charge-injectin devices" PROG. INORG. CHEM. (1997), 44(MOLECULAR LEVEL ARTIFICIAL PHOTOSYNTHETIC MATERIALS), 345-393 CODEN: PIOCAR;ISSN: 0079-6379, XP002108197 see the whole document ---	1,26
A	WO 92 21976 A (FISONS PLC) 10 December 1992 see the whole document ---	1,26
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1999	21/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 364 797 A (OLSON DAVID H ET AL) 15 November 1994 see column 1, line 5 - column 4, line 56	1,26
A	EP 0 596 421 A (HOFFMANN LA ROCHE) 11 May 1994 see abstract	1,26
A,P	US 5 874 047 A (FROHNHOFF STEPHAN ET AL) 23 February 1999 see abstract	1,26
A	WO 96 00198 A (PENTH BERND) 4 January 1996 cited in the application see the whole document	1,26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/00999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5585646 A	17-12-1996	US 5506420 A	09-04-1996
WO 9221976 A	10-12-1992	EP 0643833 A	22-03-1995
		JP 6507709 T	01-09-1994
US 5364797 A	15-11-1994	NONE	
EP 0596421 A	11-05-1994	CA 2108705 A	07-05-1994
		JP 6265553 A	22-09-1994
US 5874047 A	23-02-1999	DE 4427921 A	15-02-1996
		CA 2196895 A	22-02-1996
		WO 9605512 A	22-02-1996
		EP 0775314 A	28-05-1997
		JP 10504388 T	28-04-1998
WO 9600198 A	04-01-1996	DE 4421978 A	04-01-1996
		DE 4437767 A	25-04-1996
		DE 4439722 A	15-05-1996
		EP 0766657 A	09-04-1997
		US 5885657 A	23-03-1999

CLAIMS

1. A biosensor, comprising a surface, a nanocrystalline metal oxide semiconductor film at least partially covering said surface and at least one protein immobilised on at least a portion of said film.

2. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is titanium dioxide.

3. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is zinc oxide.

4. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is zirconium dioxide.

5. A biosensor according to any of claims 1 ~~to 4~~, wherein said film is a bioderivitised film to which said at least one protein is immobilised.

6. A biosensor according to any of claims 1 ~~to 5~~, wherein said film forms an array on said surface.

7. A biosensor according to claim 6, wherein different proteins are bound to different portions of said array.

8. A biosensor according to any of claims 1 ~~to 7~~, further comprising a pH-sensitive dye partially covering said surface.

9. A biosensor according to any of claims 1 ~~to 8~~, wherein said biosensor is an electrochemical biosensor, and further comprising an electrical circuit electrically connected to said film, said circuit comprising a detector for monitoring changes in the current or voltage in said circuit produced by an electrochemical reaction.

10. A biosensor according to any of claims 1 ~~to 8,~~ wherein said biosensor is an optical biosensor, and further comprising an optical sensor for monitoring a reaction by sensing the interaction of electromagnetic radiation with the molecules present.

11. An optical biosensor according to claim 10, wherein said at least one protein is a fluorescent or fluorophore labelled protein, said film is optically transparent, and further comprising a light source and control electronics for calculating concentrations from the output of said optical sensor.

12. A biosensor according to any of claims 1 to 8, further comprising an electrical circuit electrically connected to said film, and an optical sensor.

13. A biosensor according to claim 12, wherein said at least one protein is such as to be electrochemically or photochemically switched to a sensing state by oxidation or reduction, the results of the sensing reaction being measured optically or electrically.

14. A biosensor according to any of claims 1 to 13, wherein said biosensor further comprises a power supplying element.

15. A biosensor according to claim 14, wherein said power supplying element comprises a photoelectric element operable to supply power in response to electromagnetic radiation.

16. A biosensor according to claim 15, wherein a portion of said film forms said photoelectric element.

17. A method of manufacturing a biosensor, comprising the steps of covering at least a portion of a surface with a film of a nanocrystalline semiconductor, contacting said preformed film with a protein such as to immobilise said protein on said film.

18. A method of manufacturing a biosensor according to claim 17, wherein said film is applied to said surface by screen printing.

19. A method of manufacturing a biosensor according to claim 17 ~~or 18~~, wherein said film is contacted with a protein by immersion of said at least partially covered surface in an aqueous solution of said protein.

20. A method of manufacturing a biosensor according to claim 17 ~~or 18~~, wherein said protein is deposited on said film using a gridding robot or other dispensing device such as an ink-jet printer.

21. A method of manufacturing a biosensor according to any of claims 17 ~~to 20~~, wherein the temperature at which said film is contacted with said protein is substantially 4 °C.

CLAIMS

1. A biosensor, comprising a surface, a nanocrystalline metal oxide semiconductor film at least partially covering said surface and at least one protein immobilised on at least a portion of said film.

2. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is titanium dioxide.

3. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is zinc oxide.

4. A biosensor according to claim 1, wherein said nanocrystalline metal oxide is zirconium dioxide.

5. A biosensor according to ~~any of claims 1 to 4~~ claim 1, wherein said film is a bioderivitised film to which said at least one protein is immobilised.

6. A biosensor according to ~~any of claims 1 to 5~~, wherein said film forms an array on said surface.

7. A biosensor according to claim 6, wherein different proteins are bound to different portions of said array.

8. A biosensor according to ~~any of claims 1 to 7~~ claim 1, further comprising a pH-sensitive dye partially covering said surface.

9. A biosensor according to ~~any of claims 1 to 8~~ claim 1, wherein said biosensor is an electrochemical biosensor, and further comprising an electrical circuit electrically connected to said film, said circuit comprising a detector for monitoring changes in the current or voltage in said circuit produced by an electrochemical reaction.

10. A biosensor according to ~~any of claims 1 to 8~~ Claim 1, wherein said biosensor is an optical biosensor, and further comprising an optical sensor for monitoring a reaction by sensing the interaction of electromagnetic radiation with the molecules present.

11. An optical biosensor according to claim 10, wherein said at least one protein is a fluorescent or fluorophore labelled protein, said film is optically transparent, and further comprising a light source and control electronics for calculating concentrations from the output of said optical sensor.

12. A biosensor according to ~~any of claims 1 to 8~~ Claim 1, further comprising an electrical circuit electrically connected to said film, and an optical sensor.

13. A biosensor according to claim 12, wherein said at least one protein is such as to be electrochemically or photochemically switched to a sensing state by oxidation or reduction, the results of the sensing reaction being measured optically or electrically.

14. A biosensor according to ~~any of claims 1 to 13~~ Claim 1, wherein said biosensor further comprises a power supplying element.

15. A biosensor according to claim 14, wherein said power supplying element comprises a photoelectric element operable to supply power in response to electromagnetic radiation.

16. A biosensor according to claim 15, wherein a portion of said film forms said photoelectric element.

17. A method of manufacturing a biosensor, comprising the steps of covering at least a portion of a surface with a film of a nanocrystalline semiconductor, contacting said preformed film with a protein such as to immobilise said protein on said film.

18. A method of manufacturing a biosensor according to claim 17, wherein said film is applied to said surface by screen printing.

19. A method of manufacturing a biosensor according to claim 17 ~~or 18~~, wherein said film is contacted with a protein by immersion of said at least partially covered surface in an aqueous solution of said protein.

20. A method of manufacturing a biosensor according to claim 17 ~~or 18~~, wherein said protein is deposited on said film using a gridding robot or other dispensing device such as an ink-jet printer.

21. A method of manufacturing a biosensor according to claim 17 ~~any of claims 17 to 20~~, wherein the temperature at which said film is contacted with said protein is substantially 4 °C.